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Evaluation of glucose utilization capacity of bioactivity guided fractions of *Hybanthus enneaspermus* and *Pedalium murex* in isolated rat hemidiaphragm

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ABSTRACT

Objective: To investigate glucose utilization capacity of bioactivity guided fractions of *Hybanthus enneaspermus* (*H. enneaspermus*) and *Pedalium murex* (*P. murex*) in isolated rat hemidiaphragm. **Methods:** Dried coarsely powdered plant material was extracted in ethanol using soxhlation technique, further extract was fractionated using solvents of varying polarity. Glucose utilization capacity of bioactivity guided fractions using isolated rat hemidiaphragm was performed in the present study. **Results:** The entire tested fraction showed increased glucose uptake capacity, and was found to be maximum in case of chloroform fraction of *P. murex* extract (CHPM) which was quite comparable to standard insulin ($P<0.05$). **Conclusions:** *In vitro* glucose uptake by hemidiaphragm study showed increased utilization of the glucose by hemidiaphragm in the presence of different fractions. From these findings we can conclude that that different fraction of both plant materials had some extra pancreatic mechanism like glucose uptake by peripheral tissues.

1. Introduction

Diabetes mellitus is a chronic metabolic disorder affecting a large number of populations in the world and mainly characterized by hyperglycemia, altered metabolism of lipids, carbohydrates and proteins. Diabetes mellitus affects human body in terms of physical, psychological and social health[1,2]. Diabetes mellitus occurs due to results from an insufficient insulin secretion, insulin action or both. Type 2 diabetes mellitus or non-insulin-dependent diabetes mellitus is the most common form of diabetes mellitus, in which body does not produce enough insulin or defect in the utilization of insulin[3,4]. The drugs which lower the blood sugar level are known as hypoglycemic agents. They can be categorized into insulin and insulin preparations, which are employed only parenterally and oral hypoglycemic drug such as sulfonylureas, biguanides and glinides[1,5]. Traditional medicines from readily available medicinal plants offer great potential for the discovery of new antidiabetic drugs[6]. Antihyperglycemic activity of the plants are mainly due to their ability to restore the function of pancreatic tissues by causing an increase in insulin output or inhibit the intestinal absorption of glucose or facilitation of metabolites in insulin dependent processes. Most plants contain glycosides, alkaloids, terpenoids, flavonoids, cartenoids, *etc*., that are frequently implicated as having antidiabetic effect[7].

Hybanthus enneaspermus (Violaceae) (*H. enneaspermus*) is an herb distributed in the tropical and subtropical regions of the world. The plant has aphrodisiac, demulcent, tonic, anticonvulsant, antimalarial and diuretic properties. It is used for the treatment of various disorders including urinary infections, diarrhoea, leucorrhoea, dysuria, inflammation and male sterility. Pharmacologically, this plant has been validated for antimicrobial, antiinflammatory, antitussive, antiplasmodial, anticonvulsant, antidiabetic, aldose reductase

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inhibitory activity and antioxidant activities *etc*[8].

Pedalium murex (Pedaliaceae) (*P. murex*) is mainly used as tonic, aphrodisiac, appetisers and is useful in strangury, urinary discharges, vesicular calculi, cough, asthma, pain, skin diseases, heart troubles, piles and leprosy. The leaves are cooked and eaten as a vegetable. The whole plant is used as diuretic, dysuria, anti-biliousness, antileaves, demulcent, emmenagogue and tonic for health and vigor. Pharmacologically, this plant have been validated for antiulcerogenic, nephroprotective, hypolipidemic, aphrodisiac, antioxidant, antimicrobial and insecticidal properties[9].

2. Material and methods

2.1. Procurement of plant material and authentication

The whole plant material of *H. enneaspermus* was procured from herbal vendors in Chennai, and was identified by the chief botanist TAMPCOL Anna Hospital Chennai, India. The fruit of *P. murex* was procured from herbal market in Varanasi, and was identified by Prof. S. D. Dubey, Department of Dravyaguna, Institute of Medical Science, Banaras Hindu University, Varanasi, India.

2.2. Preparation of plant extract and its fractions

Dried coarsely powdered plant material of *H. enneaspermus* and *P. murex* were extracted in ethanol using soxhlation technique. The extract was filtered and concentrated in a rotavapor to get crude ethanolic extract. Further the extract was fractionated using solvents of varying polarity (petroleum ether, chloroform and water).

2.3. Glucose utilization by isolated rat hemi Diaphragm

Glucose utilization by rat hemi-diaphragm was estimated using regular insulin (Biocon Ltd.) as a positive control group and rats hemidiaphragm were used for the assay. Glucose uptake per gram of tissue was calculated as the difference between the initial and final glucose content in the incubated medium[10,11].

2.3.1. Experimental animals

Male Charles Foster rats (150-250 g) were used in the present study. They were housed in polypropylene cages under standard laboratory conditions [12 h light/12 h darkness, (21±2) ℃]. The experimental study was approved by Institutional Animal Ethical Committee of Institute of Medical Sciences, Banaras Hindu University, Varanasi, India.

2.3.2. Glucose uptake by isolated rat hemi-diaphragm

For determination of glucose uptake by rat hemi-diaphragm, Group Ⅰ served as a control containing 2 mL of Tyrode solution with 2% glucose, Group Ⅱ contained 2 mL Tyrode solution with 2% glucose and regular insulin 0.62 mL of 0.4 units per mL solution. Group Ⅲ contained 2 mL Tyrode solution with 2% glucose and 1.38 mL of sample and the Group Ⅳ contained 2 mL Tyrode solution with 2% glucose and regular insulin 0.62 mL of 0.4 units per mL solution and 1.38 mL of plant fraction. Volumes of all the test tubes were made up to 4 mL with distilled water.

The diaphragm was dissected out quickly and divided into two equal parts after sacrificing them by cervical dislocation. Two diaphragms from the same animal were not used for the same set of experiment. The hemi-diaphragms were placed in test tubes and incubated for 30 min at 37 ℃ in an atmosphere of 100% oxygen with shaking at 140 cycles/min. Glucose uptake per gram of tissue was calculated as the difference between the initial and final glucose content in the incubated medium[10,11].

2.3.3. Statistical analysis

Results were expressed as mean value ± standard error mean (SEM) of triplicate. One-ways ANOVA followed by Bonferroni post test was performed for evaluation of all data. GraphPad Prism (Version 4) software was used for all statistical analysis and $P<0.05$ was considered as significance.

3. Result

Several studies have demonstrated the beneficial effects of *H. enneaspermus* and *P. murex*. Based upon the ethnopharmacological reports of *H. enneaspermus* and *P. murex* being used for the treatment of diabetes in the traditional practice, protective effects of both plant *i.e. H. enneaspermus* and *P. murex* on diabetes mellitus and cataract have been investigated in our previous study[12-15]. Phytochemical analysis, *in vitro* antioxidant and aldose reductase inhibitory activity of crude ethanolic extracts and different fractions were also investigated in our previous study.

Table 1

Effect of different fractions of *H. enneaspermus* on glucose utilization by isolated rat hemi-diaphragm (n=6).

Group	Treatment	Incubation medium	Glucose uptake (mg/g/30 min)
1	Control	Tyrode solution with glucose (2%)	14.55±0.38
2	Insulin	Tyrode solution with glucose (2%) + Insulin (0.25 IU/mL)	28.30±0.68[a]
3	EEHE	Tyrode solution with glucose (2%) + plant extract (25 mg/mL)	21.92±0.65
4	Insulin + EEHE	Tyrode solution with glucose (2%) + Insulin (0.25 IU/mL) + plant extract (25 mg/mL)	24.31±2.17
5	CHHE	Tyrode solution with glucose (2%) + plant extract (25 mg/mL)	18.81±3.09
6	Insulin + CHHE	Tyrode solution with glucose (2%) + Insulin (0.25 IU/mL) + plant extract (25 mg/mL)	20.23±2.82
7	PTHE	Tyrode solution with glucose (2%) + plant extract (25 mg/mL)	17.50±2.91
8	Insulin + PTHE	Tyrode solution with glucose (2%) + Insulin (0.25 IU/mL) + plant extract (25 mg/mL)	20.03±3.24
9	AQHE	Tyrode solution with glucose (2%) + plant extract (25 mg/mL)	27.19±0.83
10	Insulin + AQHE	Tyrode solution with glucose (2%) + Insulin (0.25 IU/mL) + plant extract (25 mg/mL)	19.40±0.76

EEHE – Ethyl acetate fraction of *H. enneaspermus*, CHHE– Chloroform fraction of *H. enneaspermus*, PTHE– Petroleum ether fraction of *H. enneaspermus*, AQHE – Aqueous fraction of *H. enneaspermus*. Values are mean±SD from 3 experiments. a: compared with control group, b: compared with insulin, P<0.05 was considered as significant (One-way ANOVA followed by Bonferroni post test).

Table 2

Effect of different fractions of *P. murex* on glucose utilization by isolated rat hemi-diaphragm (n=6).

Group	Treatment	Incubation medium	Glucose uptake (mg/g/30 min)
1	Control	Tyrode solution with glucose (2%)	14.55±0.38
2	Insulin	Tyrode solution with glucose (2%) + Insulin (0.25 IU/mL)	28.30±0.68[a]
3	EEPM	Tyrode solution with glucose (2%) + plant extract (25 mg/mL)	26.17±0.30[a]
4	Insulin + EEPM	Tyrode solution with glucose (2%) + Insulin (0.25 IU/mL) + plant extract (25 mg/mL)	23.39±2.78
5	CHPM	Tyrode solution with glucose (2%) + plant extract (25 mg/mL)	19.76±2.75
6	Insulin + CHPM	Tyrode solution with glucose (2%) + Insulin (0.25 IU/mL) + plant extract (25 mg/mL)	21.62±2.28[a]
7	PTPM	Tyrode solution with glucose (2%) + plant extract (25 mg/mL)	17.48±2.13
8	Insulin + PTPM	Tyrode solution with glucose (2%) + Insulin (0.25 IU/mL) + plant extract (25 mg/mL)	21.03±1.06
9	AQPM	Tyrode solution with glucose (2%) + plant extract (25 mg/mL)	26.99±1.46
10	Insulin + AQPM	Tyrode solution with glucose (2%) + Insulin (0.25 IU/mL) + plant extract (25 mg/mL)	22.38±1.94

EEPM – Ethyl acetate fraction of *P. murex*, CHPM – Chloroform fraction of *P. murex*, PTPM– Petroleum ether fraction of *P. murex*, AQPM – Aqueous fraction of *P. murex*. Values are mean±SD from 3 experiments. a: compared with control group, b: compared with insulin, P<0.05 was considered as significant (One-way ANOVA followed by Bonferroni post test).

4. Discussion

Previous studies revealed that, the extract and different fractions contain significant amount of polyphenols and exhibited good antioxidant and aldose reductase inhibitory potential[16-19]. So, keeping these things in the mind we further extended our study to evaluate *in vitro* glucose utilization capacity of different fractions *i.e.* pet-ether, chloroform, and aqueous fraction of *H. enneaspermus* and *P. murex* using isolated rat hemi-diaphragm result showed that the chloroform fraction of *P. murex* extract (CHPM) showed more significant glucose utilization by rat hemi-diaphragm compared to the other fractions *i.e* petroleum ether and aqueous fraction (Table 1 and Table 2). Moreover in all the fractions, chloroform and aqueous fraction showed more pronounced effect compared to the petroleum ether fractions.

In vitro glucose uptake by hemidiaphragm study showed increased utilization of the glucose by hemidiaphragm in the presence of different fraction, suggesting that the fractions had some extra pancreatic mechanism like glucose uptake by peripheral tissues. The above contention is similar to the mode of action which has already been reported in literature[10-12,15]. Moreover CHPM showed more pronounced effect in the presence of insuline compared to insulin and positive control group signified their drug intraction between fraction and insulin.

Conflict of interest statement

The authors report no conflict of interest.

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Refrences

[1] Warjeet Singh L. Traditional medicinal plants of Manipur as anti-diabetics. *J Med Plants Res* 2011; **5**(5): 677-687.

[2] Pareek H, Sharma S, Khajja BS, Jain K, Jain GC. Evaluation of hypoglycemic and anti hyperglycemic potential of *Tridax procumbens* (Linn.). *BMC Complement Altern Med* 2009; **9**: 48.

[3] Balaraman AK, Singh J, Dash S, Maity TK. Antihyperglycemic and hypolipidemic effects of *Melothria maderaspatana* and *Coccinia* indica in streptozotocin induced diabetes in rats. *Saudi Pharm J* 2010; **18**(3): 173-178.

[4] Li WL, Zheng HC, Bukuru J, De Kimpe N. Natural medicines used in the traditional Chinese medical system for therapy of diabetes mellitus. *J Ethnopharmacol* 2004; **92**(1): 1-21.

[5] Saxena A, Vikram NK. Role of selected Indian plants in management of type 2 diabetes, a review. *J Altern Complement Med* 2004; **10**(2): 369-378.

[6] Malviya N, Jain S, Malviya S. Antidiabetic potential of medicinal plants. *Acta Pol Pharm* 2010; **67**(2): 113-118.

[7] Jung M, Park M, Lee HC, Kang YH, Kang ES, Kim SK. Anti-diabetic agents from medicinal plants. *Curr Med Chem* 2006; **13**(10): 1203-1218.

[8] Patel DK, Prasad SK, Sairam K, Hemalatha S. *Hybanthus enneaspermus* (Linn.) F. Muell: a concise report on its phytopharmacological aspects. *Chin J Nat Med* 2011 [Accepted] [Springer].

[9] Patel DK, Laloo D, Kumar R, Hemalatha S. *Pedalium murex* Linn.: an overview of its phytopharmacological aspects. *Asian Pac J Trop Med* 2011; **4**(9): 748-755.

[10] Hemalatha S, Sachdeva N, Wahi AK, Singh PN, Chansouria JPN. Effect of aqueous extract of fruits of *Withania coagulans* on glucose utilization by rat hemidiaphragm. *Indian J Nat Prod* 2005; **21**: 20-21.

[11] Ravi S, Sadashiva CT, Tamizmani T, Balasubramanian T, Rupeshkumar M, Balachandran I. *In vitro* glucose uptake by isolated rat hemi-diaphragm study of *Aegle marmelos* Correa root. *Bangladesh J Pharmacol* 2009; **4**: 65-68.

[12] Patel DK, Kumar R, Prasad SK, Sairam K, Hemalatha S. Antidiabetic and *in vitro* antioxidant potential of *Hybanthus enneaspermus* linn f. muell in streptozotocin-induced-diabetic rats. *Asian Pac J Trop Biomed* 2011; **1**(4): 316-322.

[13] D.K. Patel, S.K. Prasad, S. Krishnamurthy, S. Hemalatha. Evaluation of antidiabetic and antioxidant potential of *Pedalium murex* linn. fruit in streptozotocin induced-diabetic rats. *Chin J Integr Med* 2011[Accepted] [Springer].

[14] Patel DK, Prasad SK, Kumar M, Sairam K, Hemalatha S. *Pedalium murex* linn fruit: Antidiabetic, antioxidant and hypolipidemic activity in streptozotocin-nicotinamide induced (Type 2) diabetic rats. *Chin J Integr Med* 2012 [Accepted] [Springer].

[15] Patel DK, Laloo D, Sairam K, Hemalatha S. Antidiabetic, antioxidant and lipid-lowering activity of *Hybanthus enneaspermus* (L.) F. Mull. in streptozotocin-nicotinamide induced Type-2 diabetic rats. *Chin J Integr Med* 2012 [Accepted] [Springer].

[16] Patel DK, Kumar R, Prasad SK, Hemalatha S. *Pedalium murex* Linn (Pedaliaceae) fruits: A comparative antioxidant activity of its different fractions. *Asian Pac J Trop Biomed* 2011; **1**(5): 395-400.

[17] Patel DK, Kumar R, Laloo D, Hemalatha S. Evaluation of phytochemical and antioxidant activities of the different fractions of *Hybanthus enneaspermus* (Linn.) F. Muell. (Violaceae). *Asian Pac J Trop Med* 2011; **4**(5): 412-420.

[18] Patel DK, Kumar R, Kumar M, Sairam K, Hemalatha S. Evaluation of aldose reductase inhibitory potential of different fraction of *Hybanthus enneaspermus* linn f. muell. *Asian Pac J Trop Biomed* 2012; **2**(2): 134-139.

[19] Patel DK, Kumar R, Sairam K, Hemalatha S. Aldose reductase inhibitory activity of different fraction of *Pedalium murex* Linn fruits in rat lens. *Chin J Integr Med* 2012. [Accepted] [Springer].